# ISOLATE II
## RNA Micro Kit

Product Manual

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# **ISOLATE II** RNA Micro Kit

Table of Contents

# 1. KIT CONTENTS

| COMPONENT | 10 Preps | 50 Preps |
|---|---|---|
| ISOLATE II Filters (violet) | 10 | 50 |
| ISOLATE II RNA Micro Columns (blue) & Collection Tubes | 10 | 50 |
| Collection Tubes (2 mL) | 30 | 150 |
| Collection Tubes (1.5 mL) | 10 | 50 |
| Lysis Buffer RLY* | 6 mL | 25 mL |
| Wash Buffer RW1* | 2 x 1 mL | 15 mL |
| Wash Buffer RW2† (concentrate) | 6 mL | 12 mL |
| Membrane Desalting Buffer MEM* | 10 mL | 10 mL |
| Reaction Buffer for DNase I RDN | 7 mL | 7 mL |
| DNase I, RNase-free (lyophilized) | 1 vial | 1 vial |
| Carrier RNA | 300 µg | 300 µg |
| Reducing Agent TCEP | 14 mg | 3 x 14 mg |
| RNase-free Water | 13 mL | 13 mL |
| Bench Protocol Sheet | 1 | 1 |

** Contains a guanidine salt. Not compatible with disinfectants containing bleach or acidic solutions. See safety information in section 4.*

*† Before use, add indicated volume of 96 - 100% ethanol and mark wash buffer bottle label.*

# 2. DESCRIPTION

The ISOLATE II RNA Micro Kit is specially designed for isolation of high-quality total cellular RNA from small amounts of samples (from a single cell to tissue). Suitable starting materials include microdissected cryosections, fine needle aspirates, pellets of cultured cells and flow cytometer sorted cells.

Biological samples are first lysed and homogenized in the presence of guanidinium thiocyanate, a chaotropic salt which immediately deactivates endogenous RNases to ensure purification of intact RNA. After homogenization, ethanol is added to the sample. The sample is then processed through a spin column containing a silica membrane to which the RNA binds. Genomic DNA contamination is removed by an on-column DNase I digestion during the preparation. Any impurities such as salts, metabolites and cellular components are removed by simple washing steps with two different buffers. High quality purified total RNA is then eluted in a small volume of RNase-free water. The highly concentrated RNA is ready-to-use for a wide variety of applications e.g. qPCR, next generation sequencing, Northern blotting, primer extension, array technology and RNase protection assays.

The ISOLATE II RNA Micro Kit allows convenient processing of multiple samples in < 40 min without needing laborious methods such as CsCl ultracentrifugation and handling toxic chemicals e.g. phenol/chloroform.

Please read this manual carefully to familiarize yourself with the ISOLATE II RNA Micro protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

# 3. STORAGE

Store lyophilized DNase I (RNase-free), Reducing Agent TCEP and Carrier RNA at 4°C on arrival (stable for up to 1 year). Reconstituted DNase I working solution is stable for 6 months at -20°C. All other kit components should be stored at room temperature (18 - 25°C) and are stable for up to 1 year. Storage at lower temperatures may cause precipitation of salts.

# 4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Buffers RLY, RW1 and MEM contain guanidinium thiocyanate. This chemical is harmful when in skin contact, inhaled or ingested.

**CAUTION:** Do not add bleach directly to solutions or sample preparation waste containing guanidine salts. Reactive compounds and toxic gases can form. Clean with a suitable laboratory detergent and water if liquid from these buffers are spilt.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

# 5. PRODUCT SPECIFICATIONS

The ISOLATE II RNA Micro Kit is specially designed for the rapid and efficient isolation of extremely pure total RNA from very small samples (up to 5 x $10^5$ cells or 5 mg tissue). The novel column design allows elution of highly concentrated RNA in very small volumes (5 - 30 µL) and is suited for a wide variety of applications e.g. qPCR and microarrays. The preparation time is approx. 40 min for 12 preps. The isolated RNA is of high-purity ($A_{260}/A_{280}$ ratio: > 1.9) and high-integrity (RIN > 9) for high-quality small samples (see below).

## ISOLATE II RNA MICRO SPIN COLUMN SPECIFICATIONS

| | |
|---|---|
| Max. binding capacity | 110 µg RNA |
| RNA size distribution | > 200 nucleotides |
| $A_{260}/_{280}$ ratio* | 1.9 - 2.1 |
| Typical RIN (RNA integrity number)† | > 9 |
| Elution volume | 5 - 30 µL |
| Max. amount of starting material<br>Cultured cells (human/other mammalian)<br>Tissue (human/other mammalian) | <br>5 x $10^5$<br>5 mg |

** Typically, the $A_{260}/A_{280}$ ratio exceeds 1.9, indicating excellent RNA purity.*

*† Agilent 2100 Bioanalyzer (RNA 6000 assay). RNA integrity is highly dependent on sample quality.*

## Typical RNA Yield

RNA yield is dependent on sample type, quality and amount. Table 1 shows expected RNA yields from a number of different sources. It is important to use the correct amount of starting material in order to obtain optimal RNA yield and purity.

**TABLE 1: TYPICAL YIELDS OF TOTAL RNA WITH ISOLATE II RNA MICRO KIT**

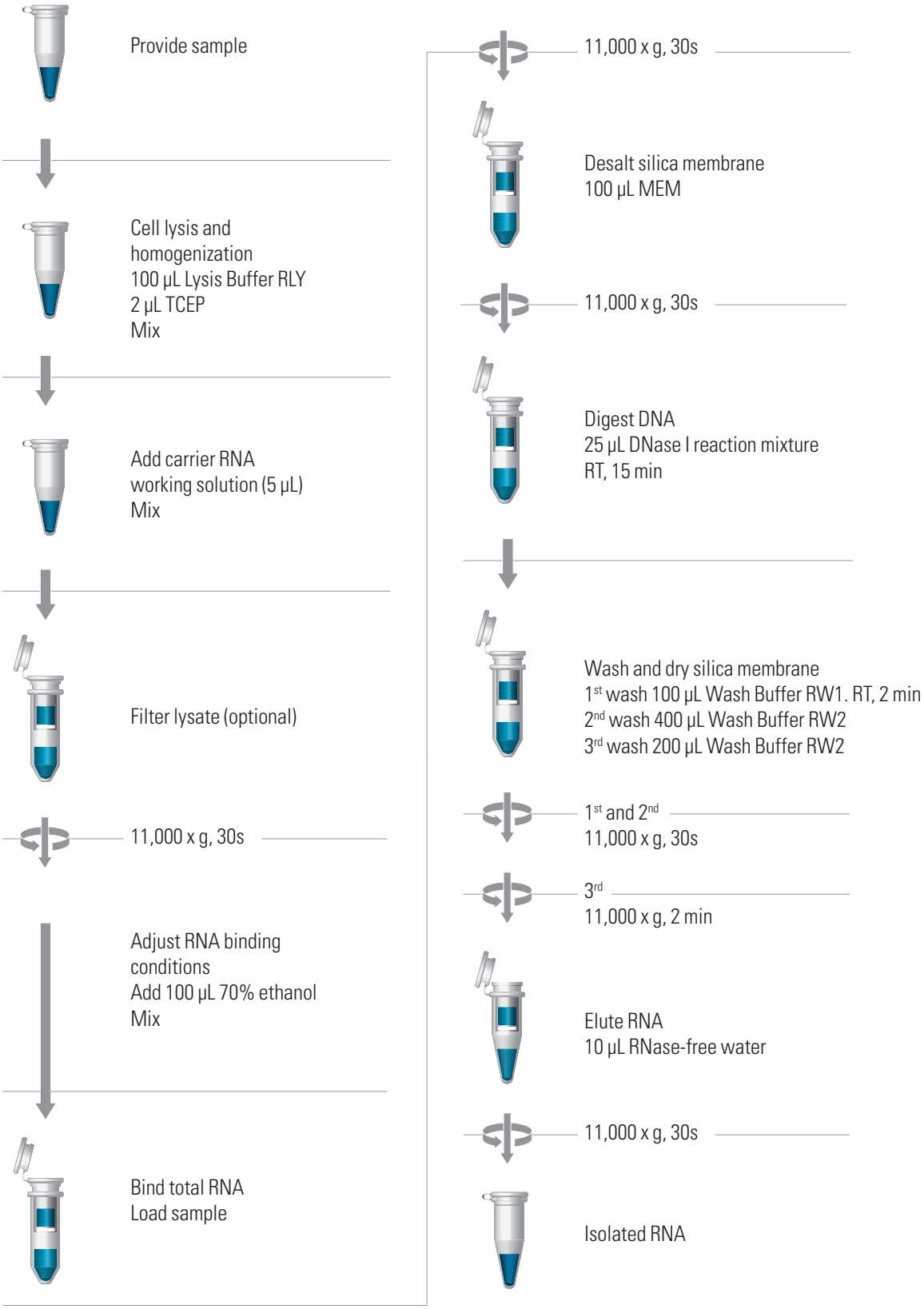
| SAMPLE TYPE | SOURCE | TOTAL RNA YIELD* (µg) |
|---|---|---|
| Mammalian cell cultures (5x$10^5$ cells) | NIH/3T3 | 5 |
| | HeLa | 7.5 |
| *Mouse/rat tissues (5 mg)* | Liver | 12 |
| | Kidney | 8 |
| | Spleen | 12 |
| | Brain | 3 |
| | Thymus | 15 |

** Yields can vary depending on factors such as species, stage of development and culture conditions.*

The following components are also included in the kit:

- ISOLATE II Filters for homogenization and reduction of lysate viscosity.
- Reducing Agent TCEP (tris(2-carboxyethyl)phosphine). TCEP has the advantages of being odorless, more powerful, more stable and less toxic than other commonly used reducing agents.
- Carrier RNA (poly-A RNA: poly(A) potassium salt) to improve RNA recovery from very small samples.
- DNase I (RNase-free) for eliminating genomic DNA contamination by on-column digestion or by digestion in solution (for the most sensitive applications).

## Total RNA Isolation



# 6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves. Please consult the relevant MSDS from the product supplier for further information.

- 70% ethanol† (to adjust RNA binding conditions)
- 96 - 100% ethanol† (for Wash Buffer RW2)
- Equipment for sample disruption and homogenization (see section 7.2). One or more of the following are required depending on chosen method.
  - PBS and trypsin
  - Needle and syringe (both RNase-free)
  - Mortar and pestle
  - Rotor-stator or bead-mill homogenizer
  - Vortex mixer
- Liquid nitrogen
- Microcentrifuge tubes (1.5 mL)
- Sterile RNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)

† *Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone. Make 70% ethanol up with nuclease-free molecular biology grade water.*

# 7. IMPORTANT NOTES

The ISOLATE II RNA Micro Kit purification procedures can be performed at room temperature. Handle the eluted RNA carefully to avoid contamination by RNases, often found on labware, fingerprints and dust. For optimal RNA stability, keep RNA frozen at -20°C for short-term or -80°C for long-term storage. When working with RNA samples in downstream applications, keep the RNA solution on ice.

See hints and tips on working with RNA at www.bioline.com/isolate.

## 7.1 HANDLING AND STORING STARTING MATERIALS

RNA is not protected against digestion until the sample is flash frozen or disrupted in the presence of RNase inhibiting or denaturing reagents. Samples should be flash frozen in liquid nitrogen immediately, stored at -80°C or processed as soon as possible. Following disruption and homogenization in Lysis Buffer RLY/TCEP, samples can be kept at -80°C for up to one year, at 4°C for up to 24 hours or up to several hours at room temperature. Frozen samples are stable for up to 6 months. Frozen samples in Lysis Buffer RLY/TCEP should be thawed slowly before starting the isolation procedure.

## 7.2 DISRUPTING AND HOMOGENIZING STARTING MATERIALS

For all RNA purification procedures, efficient disruption and homogenization of starting material is essential. To release all RNA contained in a sample, total disruption of cell walls, plasma membranes and organelles must occur. Incomplete disruption results in reduced RNA yields. Homogenization reduces lysate viscosity following disruption. Incomplete homogenization results in inefficient binding of RNA to the silica membrane and therefore reduced RNA yields.

### Cells grown in suspension

Centrifuge an appropriate number of cells and remove all supernatant by aspiration. Lyse directly by adding Lysis Buffer RLY.

***Refer to step 2 of the standard protocol (see section 8.1).***

### Cells grown in a monolayer

Remove the cell-culture medium completely. Incomplete removal of the medium will inhibit lysis of the cells and compromise the efficiency of RNA isolation. Immediately add Lysis Buffer RLY to the cell-culture plates.

***Refer to step 2 of the standard protocol (see section 8.1)***

### Trypsinization of adherent cells

To trypsinize adherent growing cells, first aspirate the cell-culture medium. Add an equal volume of PBS to wash the cells and aspirate excess liquid. Add 0.1 - 0.3% trypsin in PBS to the washed cells. Incubate until cells are detached. Add culture medium and transfer cells to an appropriate tube (not supplied). Pellet cells by centrifugation (300 x g, 5 min). Remove supernatant and add Lysis Buffer RLY to the cell pellet.

### Disruption and homogenization using lysis buffer and vortexing

The simple addition of Lysis Buffer RLY and subsequent vortexing is usually sufficient to disrupt and homogenize up to $10^4$ cultured cells as well as laser captured cells, or microdissected samples.

### Disruption using a mortar and pestle

An RNase-free mortar and pestle can be used in combination with liquid nitrogen to disrupt and lyse frozen or fibrous tissue samples, which are often solid. Grind the frozen tissue into a fine powder and add liquid nitrogen as necessary. It is important to ensure the sample does not thaw during or after grinding. Then transfer tissue powder into a liquid nitrogen cooled tube and allow liquid nitrogen to evaporate. Add Lysis Buffer RLY with reducing agent TECP to powdered tissue and mix immediately. Homogenize sample with an ISOLATE II Filter column (supplied). Alternatively, pass lysate through a nuclease-free 20-gauge (0.9 mm) syringe needle 5 - 10 times or until a homogeneous lysate is achieved.

***Note:*** *Due to the small size of samples to be processed with the ISOLATE II RNA Micro Kit, disruption by mortar and pestle may not always be suitable.*

### Disruption and homogenization using a rotor-stator homogenizer

Rotor-stator type tissue homogenizers can homogenize, disrupt and emulsify animal tissue samples in the presence of Lysis Buffer RLY in seconds to minutes. Homogenization time depends on sample size and toughness. Thawing of undisrupted mammalian tissue should only be performed in presence of Lysis Buffer RLY to prevent degradation of RNA by RNases. The spinning rotor disrupts and homogenizes the sample simultaneously by turbulence and mechanical shearing. Foaming can be minimized by keeping the rotor tip submerged. Select a suitably sized homogenizer: 5 - 7 mm diameter rotors can be used for homogenization in microcentrifuge tubes.

### Disruption and homogenization using bead-milling

Bead-milling disrupts the tissue samples in the presence of Lysis Buffer RLY and agitating beads. Disruption and homogenization occur simultaneously via the shearing / crushing action of the beads as they collide with the cells. Please refer to supplier guidelines for suitable disruption parameters.

## 7.3 CARRIER RNA

The ISOLATE II RNA Micro Kit contains poly-A RNA for use as Carrier RNA for optimal performance with very small samples. We recommend that Carrier RNA (20 ng) is added to the sample lysate. This may in some cases improve the recovery of total RNA, but is overall dependent on sample type, amount, and specific RNA application. The small amounts of poly-A RNA used as Carrier RNA in total RNA purification do not interfere with subsequent qPCR, even when oligo-dT is used as a primer for reverse transcription. Reverse-transcription reactions contain an excess amount of oligo-dT primers and the small amounts of poly-A used as Carrier RNA are insignificant in comparison. If a poly-A RNA isolation is to be performed post-isolation, Carrier RNA should not be added. Other types of Carrier RNA may be used instead e.g. bacterial ribosomal RNA (not supplied).

## 7.4 BUFFER PREPARATION AND PARAMETERS

Ensure 96 - 100% ethanol and 70% ethanol are available.

For all protocols prepare the following reagents:

### Preparing DNase I (RNase-free) stock solution

Reconstitute lyophilized DNase I in RNase-free water (supplied): Add 55 µL water for the 10 prep kit and 230 µL for the 50 prep kit. Incubate for 1 min at room temperature, then mix by gently swirling.

***Important note:*** *Do not vortex the reconstituted DNase I as the enzyme is particularly sensitive to mechanical denaturation. Dispense into aliquots to avoid excessive freeze-thawing. Do not freeze-thaw aliquots more than three times. Store aliquots at -20°C. The frozen working solution is stable for 6 months.*

### Preparing Wash Buffer RW2 with ethanol

Add 96 - 100% ethanol to Wash Buffer RW2 Concentrate: 24 mL for the 10 prep kit and 48 mL for the 50 prep kit.

***Important note:*** *Mark the label of the bottle to indicate ethanol was added. Store Wash Buffer RW2 at room temperature (18 - 25°C) for up to one year.*

### Preparing Reducing Agent TCEP

Add RNase-free water to TCEP: 100 µL for the 10 prep kit and 100 µL x 3 for the 50 prep kit. Incubate for several minutes at room temperature. Mix to completely dissolve the TCEP. Store at -20°C.

### Preparing Carrier RNA

Add 750 µL Lysis Buffer RLY to Carrier RNA to obtain a 400 ng/µL stock solution. Prepare a working solution prior to RNA purification: dilute stock solution 1:100 with Lysis Buffer RLY to obtain a 4 ng/µL working solution, e.g. 1 µL Carrier RNA stock solution + 99 µL Lysis Buffer RLY. Add 5 µL (20 ng) of this working solution to every lysate (see step 3 of standard protocol in section 8.1). Store stock solution at -20°C.

***Important note:*** *Do not store the working solution but prepare a fresh batch immediately before use.*

### Elution parameters

Standard kits commonly have a high default elution volume that results in weakly concentrated RNA that requires re-concentration for use in downstream applications. In contrast, the ISOLATE II RNA Micro Kit facilitates elution in a very small volume resulting in highly concentrated RNA. Elution volumes in the range of 5 - 30 µL are recommended (the default elution volume is 10 µL).

## 7.5 ELIMINATING GENOMIC DNA CONTAMINATION

Genomic DNA contamination is efficiently removed by on-column digestion with DNase I (supplied). Residual genomic DNA may however be detected in very sensitive applications e.g. probe-based qPCR. A DNase I digest in the eluate can be performed to remove even traces of contaminating DNA (see section 8.3).

# 8. PROTOCOLS

## 8.1 PURIFYING TOTAL RNA FROM MICRODISSECTED CRYOSECTIONS, LASER CAPTURED CELLS OR CULTURED CELLS

Before you start:

- Ensure TCEP, Carrier RNA, DNase I and Wash Buffer RW2 are prepared (see section 7.4).

### 1 Provide sample

Transfer sample e.g. microdissected tissue cryosection, pelleted cultured cells (up to 5 x $10^5$), or laser captured cells to a sterile 1.5 mL microcentrifuge tube (not supplied).

***See section 5 for recommended sample amounts.***

### 2 Cell lysis and homogenization

Add 100 µL Lysis Buffer RLY and 2 µL TCEP to sample and vortex vigorously (2 x 5s). If multiple samples are processed, a master mix is recommended (e.g. 1.1 mL Lysis Buffer RLY and 22 µL TCEP for 10 preps). Use 102 µL of the master mix per sample. This procedure is usually sufficient to homogenize cultured cells, laser captured cells, or microdissected tissue.

***Refer to section 7.2 for further details on homogenization methods.***

### 3 Add Carrier RNA

Add 5 µL (20 ng) Carrier RNA working solution to lysate. Mix by vortexing (2 x 5s). Briefly spin down (approx. 1s at 1000 x g) to clear the lid.

***Refer to section 7.4 for preparation of Carrier RNA working solution.***

### 4 Filter lysate (optional)

Place an ISOLATE II Filter (violet) in a 2 mL Collection Tube (supplied). Load lysate and centrifuge (30s at 11,000 x g). Discard the ISOLATE II Filter.

This step may be omitted when processing small amounts of sample, e.g. < $10^5$ cells.

### 5 Adjust RNA binding conditions

Add 100 µL ethanol (70%) to the homogenized lysate and mix by pipetting up and down 5 times.

*Alternatively, add 100 µL ethanol (70%) to the sample in a 1.5 mL microcentrifuge tube (not supplied) and mix by vortexing (2 x 5s). Spin down briefly (approx. 1s at 1000 x g) to clear lid. Pipette lysate up and down two times before loading the lysate.*

## 6 Bind RNA

Place an ISOLATE II RNA Micro Column (blue) in a Collection Tube. Load lysate onto the column and centrifuge (30s at 11,000 x g). Place column in a new Collection Tube (2 mL).

*Max. loading capacity of ISOLATE II RNA Micro Column is 600 µL. Repeat procedure if processing larger volumes.*

## 7 Desalt silica membrane

Add 100 µL Membrane Desalting Buffer (MEM) and centrifuge at 11,000 x g for 30s to dry membrane. Re-use Collection Tube.

*Salt reduces DNase I activity in step 8. If column outlet accidentally touches flow-through, discard flow-through and centrifuge again for 30s at 11,000 x g.*

## 8 Digest DNA

Prepare DNase I reaction mixture (see section 7.4) in a sterile 1.5 mL microcentrifuge tube (not supplied). For each isolation, add 3 µL reconstituted DNase I (also see section 7.4) to 27 µL Reaction Buffer for DNase I (RON). Mix by gently flicking the tube. Apply 25 µL DNase I reaction mixture directly onto the center of the silica membrane. Close lid and incubate at room temperature for 15 min.

*It is not necessary to use a new Collection Tube after the incubation step.*

## 9 Wash and dry silica membrane

**1st Wash**

- Add 100 µL Wash Buffer RW1 to the ISOLATE II RNA Micro Column. Incubate for 2 min at room temperature (RT). Centrifuge for 30s at 11,000 x g.

Place the column into a new 2 mL Collection Tube. Wash Buffer RW1 will inactivate the DNase I.

**2nd Wash**

- Add 400 µL Wash Buffer RW2 to the ISOLATE II RNA Micro Column. Centrifuge for 30s at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.

***Note:*** *Ensure residual buffer from previous steps is washed away with Wash Buffer RW2, especially if lysate has contacted the inner rim of the column during loading of the lysate onto the column. To efficiently wash the inner rim, flush it with Wash Buffer RW2.*

**3rd Wash**

- Add 200 µL Wash Buffer RW2 to the ISOLATE II RNA Micro Column. Centrifuge for 2 min at 11,000 x g to dry the membrane. Place the column into a 1.5 mL Collection Tube (supplied).

*If for any reason the liquid level in the Collection Tube during washes 1, 2 or 3 has reached the ISOLATE II RNA Micro Column after centrifugation, discard flow-through and centrifuge again.*

## 10 Elute RNA

Elute the RNA in 10 µL RNase-free water (supplied) and centrifuge at 11,000 x g for 30s.

*If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5-30 µL.*

***See section 7.4 for further details on alternative elution procedures.***

## 8.2 PURIFYING TOTAL RNA FROM TISSUE

Before you start:

- Ensure TCEP, Carrier RNA, DNase I and Wash Buffer RW2 are prepared (see section 7.4)

### 1 Supply sample

Transfer tissue sample into a sterile 1.5 mL microcentrifuge tube (not supplied). For appropriate sample amounts see section 7.2.

### 2 Tissue lysis and homogenization

Add 200 µL Lysis Buffer RLY and 4 µL TCEP to the tissue sample and vortex vigorously (2 x 5s).

Recommended methods for disruption and homogenization of tissues are a rotor-stator homogenizer or a bead-mill (see section 7.2).

To process multiple samples, preparing a master mix is recommended (e.g. 2.2 mL Lysis Buffer RLY and 44 µL TCEP for 10 preparations). Use 204 µL of the master mix per sample.

### 3 Add Carrier RNA

Add 5 µL (20 ng) Carrier RNA working solution to the lysate. Mix by vortexing (2 x 5s). Spin down briefly (approx. 1s at 1000 x g) to clear the lid.

***For preparation of Carrier RNA working solution see section 7.3.***

### 4 Filter lysate

Place ISOLATE II Filter (violet) in a 2 mL Collection Tube (supplied), load the lysate and centrifuge for 30s at 11,000 x g. This step helps reduce viscosity and clears the lysate.

***Note:*** *If a visible pellet forms, depending on sample amount and nature, transfer supernatant without any formed pellet to a new 1.5 mL microcentrifuge tube (not included).*

### 5 Adjust RNA binding conditions

Discard ISOLATE II Filter (violet) and add 200 µL ethanol (70%) to the homogenized lysate. Mix by pipetting up and down 5 times.

Alternatively, transfer flow-through into a new 1.5 mL microcentrifuge tube (not supplied), add 200 µL ethanol (70%), and mix by vortexing (2 x 5s). Spin down briefly (approx. 1s at 1000 x g) to clear the lid. Pipette lysate up and down two times before loading the lysate.

***Note:*** *After addition of ethanol a stringy precipitate may become visible. This will not affect the RNA isolation. Break apart any precipitate by mixing. Do not centrifuge the ethanolic lysate before loading it onto the column to prevent pelleting the precipitate.*

### 6 Bind RNA

For each preparation, take one ISOLATE II RNA Micro Column (blue) placed in a Collection Tube and load the lysate to the column. Centrifuge for 30s at 11,000 x g. Place the column in a new Collection Tube (2 mL).

Maximal loading capacity of ISOLATE II RNA Micro Columns is 600 µL. Repeat the procedure if larger volumes are to be processed.

## 7 Desalt silica membrane

Add 100 µL Membrane Desalting Buffer (MEM) and centrifuge at 11,000 x g for 30s to dry the membrane. Re-use Collection Tube.

***Note:*** *Salt reduces DNase I activity in the next step. If column outlet accidentally touches flow-through, discard flow-through and centrifuge again for 30s at 11,000 x g.*

## 8 Digest DNA

Prepare DNase I reaction mixture in a sterile microcentrifuge tube (not supplied): for each isolation, add 3 µL reconstituted DNase I (also see section 7.4) to 27 µL Reaction Buffer for DNase I (RDN). Mix by gently flicking the tube.

Apply 25 µL DNase I reaction mixture directly onto the center of the silica membrane of the column. Close the lid. Incubate at RT for 15 min. Re-use Collection Tube after the incubation step.

## 9 Wash and dry silica membrane

**1st Wash**

- Add 100 µL Wash Buffer RW1 to the ISOLATE II RNA Micro Column. Incubate for 2 min at RT. Centrifuge for 30s at 11,000 x g.

Place the column into a new Collection Tube (2 mL).

*Wash Buffer RW1 will inactivate the DNase I.*

**2nd Wash**

- Add 400 µL Wash Buffer RW2 to the ISOLATE II RNA Micro Column. Centrifuge for 30s at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.

**3rd Wash**

- Add 200 µL Wash Buffer RW2 to the ISOLATE II RNA Micro Column. Centrifuge for 2 min at 11,000 x g to dry the membrane. Place the column into a 1.5 mL Collection Tube (supplied).

*If for any reason the liquid level in the Collection Tube during washes 1, 2 or 3 has reached the ISOLATE II RNA Micro Column after centrifugation, discard flow-through and centrifuge again.*

## 10 Elute RNA

Elute the RNA with 10 µL RNase-free water (supplied) and centrifuge at 11,000 x g for 30s.

*If higher RNA concentrations or higher elution volumes are desired, elution volume may be varied in the range of 5-30 µL.*

***See section 7.4 for further details on alternative elution procedures.***

## 8.3 DNASE I TREATMENT OF PURIFIED RNA IN SOLUTION

The on-column DNase I digestion results in minimal residual DNA, undetectable in most downstream applications. For the most sensitive applications, DNA digestion in solution is recommended to eliminate even traces of contaminating DNA. Stringent RNase control as well as RNA repurification to remove buffer, salts, DNase I and digested DNA are needed.

### 1 Digest DNA (Reaction setup)

Prepare enzyme-buffer master mix: Add 1 µL DNase I to 10 µL Reaction Buffer for DNase I (RDN).

Add 1/10$^{th}$ volume to the eluted RNA e.g. add 1 µL of the enzyme-buffer master mix to 10 µL RNA.

Gently swirl tube to mix solution. Gently spin down (approx. 1s at 1,000 x g) to collect solution at bottom of the tube.

### 2 Sample incubation

Incubate for 10 min at 37°C.

### 3 RNA repurification

Repurify RNA with a suitable RNA cleanup procedure, e.g. ISOLATE II RNA Micro Clean-Up Kit or by ethanol precipitation.

Ethanol precipitation step

- Add 1/10$^{th}$ volume of sodium acetate (3 M, pH 5.2)
- Add 2.5 - 3 x volume of 96 - 100% ethanol to one volume of sample. Mix thoroughly.
- Precipitate for one hour or overnight at -20°C.

***Note:*** *Choose longer incubation times if the sample has a low RNA concentration. Shorter incubation times are sufficient for high RNA concentrations.*

- Centrifuge at maximum speed for 10 min.
- Wash RNA pellet with ice-cold 70% ethanol.
- Dry RNA pellet and resuspend RNA in RNase-free water.

## 8.4 CLEAN-UP AND CONCENTRATION OF RNA FROM REACTION MIXTURES

Before you start:

- Ensure Wash Buffer RW2 was prepared (section 7.4).

### 1 Supply sample

Transfer up to 300 µL sample such as prepurified RNA (e.g. phenol purified) or RNA from reaction mixtures (e.g, labeling reactions) in a sterile 1.5 mL microcentrifuge tube (not supplied).

***For appropriate sample amounts see section 5.***

### 2 Prepare lysis-binding buffer premix

For every 100 µL of sample combine 25 µL Lysis Buffer RLY with 75 µL ethanol (96 - 100%) and mix.

If processing multiple samples, the preparation of a master-premix (1 volume Lysis Buffer RLY plus 3 volumes ethanol 96 - 100%) is recommended.

### 3 Add Carrier RNA

Not required.

## 4 Filter lysate

Not required.

## 5 Adjust RNA binding conditions

Add one volume of premix to the sample (e.g. 100 µL premix to a 100 µL sample) and mix (2 x 5s). If necessary, spin down briefly (approx. 1s 1000 x g) to clear the lid.

## 6 Bind RNA

Place an ISOLATE II RNA Micro Column (blue) in a Collection Tube and load the lysate onto the column. Centrifuge for 30s at 11,000 x g.

For samples > 300 µL, load in two steps.

Place column in a new 2 mL Collection Tube.

*For highly demanding applications, the recovery rate can be increased as follows: Centrifuge 30s at 2,000 x g prior to centrifugation for 30s at 11,000 x g.*

## 7 Desalt silica membrane

Not required.

## 8 Digest DNA

Not required.

## 9 Wash and dry silica membrane

**1st Wash**

- Add 400 µL Wash Buffer RW2 to the ISOLATE II RNA Micro Column. Centrifuge for 30s at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.

**2nd Wash**

- Add 200 µL Wash Buffer RW2 to the ISOLATE II RNA Micro Column. Centrifuge for 2 min at 11,000 x g to dry the membrane. Place the column into a nuclease-free Collection Tube (1.5 mL; supplied).

If for any reason the liquid level in the Collection Tube during washes 1 or 2 has reached the ISOLATE II RNA Micro Column after centrifugation, discard flow-through and centrifuge again.

## 10 Elute RNA

Elute the RNA in 10 µL RNase-free water (supplied) and centrifuge at 11,000 x g for 30s.

*If higher RNA concentrations or higher elution volumes are desired, the elution volume may be varied from 5 - 30 µL.*

***See section 7.4 for alternative elution procedures.***

# 9. TROUBLESHOOTING GUIDE

## CLOGGED SPIN COLUMN

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| Insufficient disruption or homogenization | Reduce starting material or use s larger volume of Lysis Buffer RLY. Ensure thorough disruption and use ISOLATE II Filters for homogenization of disrupted starting material. |
| Insufficient centrifugation | Increase centrifugation speed and time. |

## LOW RNA YIELD OR QUALITY

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| Insufficient disruption or homogenization | Reduce starting material or use a larger volume of Lysis Buffer RLY. Ensure thorough disruption and use ISOLATE II Filters for homogenization of disrupted starting material. |
| Sample material degraded | Store sample material properly. Use fresh material whenever possible; if not, flash-freeze sample in liquid nitrogen. Always keep samples at -80°C. Always add Lysis Buffer before thawing sample. Disrupt samples in liquid nitrogen and ensure tubes are kept chilled. |
| Incomplete elution | Incubate sample with RNase-free water for up to 5 minutes and repeat elution step. |
| Reagents not properly prepared | Add Lysis Buffer ALY to Carrier RNA to make stock and working solutions.<br>Add reducing agent TCEP to Lysis Buffer RLY.<br>Add 96 - 100% ethanol to Wash Buffer RW2 concentrate. Ethanol is required to create effective binding conditions for RNA to the silica membrane.<br>Add RNase-free water to DNase I.<br>Prepare and store reagents according to instructions given in section 7.4. |

## LOW RNA CONCENTRATION

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| High elution volume | Elute RNA with a lower volume. |
| Inappropriate handling and storing of starting material | Ensure proper handling and storage of samples. Ensure that all steps are followed quickly. |

## LOW $A_{260}/A_{280}$ RATIO

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| Guanidinium thiocyanate carryover | Carefully load lysate to ISOLATE II RNA Micro Column, avoid contamination between column and lid. Ensure residual Wash Buffer RW1 is cleaned with Wash Buffer RW2. Apply Wash Buffer RW2 to inner rim of column. |

## UNEXPECTED $A_{260}/A_{280}$ RATIO

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| Measurement falls below detection limit of spectrophotometer | To obtain a significant $A_{260}/A_{280}$ ratio, initially measured $A_{260}$ and $A_{280}$ values must be significantly above the detection limit of the spectrophotometer used. An $A_{280}$ value near the background noise of the photometer will result in unexpected $A_{260}/A_{280}$ ratios. |

## RNA DEGRADED

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| RNase contamination | Ensure an RNase-free working environment (see online hints and tips at www.bioline.com).<br>Discard any solutions contaminated with RNase during use. |

## DNA CONTAMINATION

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| Incorrect lysis | Check protocol has been followed correctly. |
| Too much starting material | Reduce amount of starting material. |
| DNase I inactive | Reconstitute lyophilized DNase I and store as recommended. |
| Oversensitive DNA detection | Use intron spanning primers if possible.<br>Use larger PCR amplicons (DNA detection probability increases with smaller PCR amplicons).<br>Use DNase I digestion in solution protocol. |

## RNA DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS

| POSSIBLE CAUSE | RECOMMENDED SOLUTION |
|---|---|
| Ethanol carryover during elution | Increase centrifugation time for ethanol removal step<br>Do not allow Wash Buffer RW2 flow through to touch column outlets after second wash |
| Salt carryover during elution | Ensure that Wash Buffers are at room temperature.<br>Washing at lower temperatures reduces efficiency of salt removal. Check both solutions for salt precipitates. Resuspend any visible precipitate by gentle warming. |
| RNA not stored optimally | Always keep eluted RNA on ice to prevent degradation by RNases.<br>Store at -20°C for short term storage or -80°C for long term storage. |

# A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@meridianlifescience.com

# B. ORDERING INFORMATION

| PRODUCT | PACK SIZE | CAT NO. |
|---|---|---|
| ISOLATE II RNA Micro Kit | 10 Preps | BIO-52074 |
| ISOLATE II RNA Micro Kit | 50 Preps | BIO-52075 |

# C. ASSOCIATED PRODUCTS

| PRODUCT | PACK SIZE | CAT NO. |
|---|---|---|
| ISOLATE II RNA Mini Kit | 10 Preps | BIO-52071 |
| ISOLATE II RNA Mini Kit | 50 Preps | BIO-52072 |
| ISOLATE II RNA Mini Kit | 250 Preps | BIO-52073 |
| ISOLATE II RNA Plant Kit | 10 Preps | BIO-52076 |
| ISOLATE II RNA Plant Kit | 50 Preps | BIO-52077 |
| RiboSafe RNase Inhibitor | 2,500 Units | BIO-65027 |

# D. PRODUCT WARRANTY AND DISCLAIMER

Meridian warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Meridian will replace free of charge any product that does not conform to the specifications. This warranty limits Meridian's liability only to the replacement of the product.

# NOTES

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**Technical Support**
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